A Computational Model For Nanoparticle Delivery Across Lymphatics

Jenny Yarmovsky, Dr. Katharina Maisel
Fischell Department of Bioengineering, University of Maryland College Park

Introduction

- Targeting lymphatics allows for therapeutic administration to the lymph nodes, where the adaptive immune response is shaped.
- Immunotherapies rely on drug accumulation in the lymph nodes in order to elicit or suppress an immune response **(Fig 1)**.
- Lymphatic targeting allows for systemic delivery while also bypassing first-pass metabolism.
- Drug delivery vehicles can be designed to optimize drug accumulation in lymph nodes by changing their properties[1].
  - Size and surface chemistry of nanoparticles can be altered to improve transport.
  - The mechanisms behind these results are poorly understood.
  - Conflicting data exists about optimal properties.
  - Computational models are a valuable tool for elucidating the relationship between nanoparticle properties and transport.
  - Models allow us to predict the effect of changing properties of the drug delivery vehicle on its transport.
  - Here, we were able to correlate nanoparticle surface chemistry with improved lymphatic transport, and identify mechanisms behind transport across lymphatic endothelial cells.

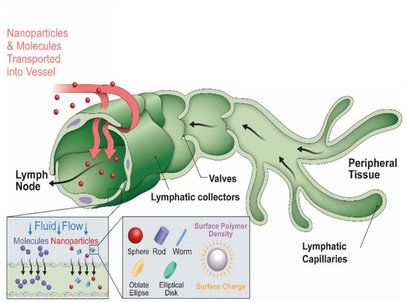

Figure 1: Lymphatic transport mechanisms Lymphatics transport materials from peripheral tissues via capillaries and collecting vessels to the lymph nodes. Lymphatics transport materials across the vessel wall via paracellular and transcellular transport routes.

Methods

Lymphatic Transport Model

- Experimental data for model development and validation was obtained using an established in vitro model for nanoparticle transport[2] **(Fig 2)**.
- Human primary LECs were seeded on transwell inserts and cultured under transmural flow for 24 hours.
- For the experiments with transport inhibitors, 100 nM Adrenomedullin and 62.5 μM of Dynasore were added to cell media.
- 100 nm polystyrene nanoparticles were generated with 100%, 50%, 25%, and 10% polyethylene glycol coverage.
- Mass-action laws were used to develop equations 1 and 2.
- n_{up} and n_{lo} represent nanoparticle concentrations in the upper and lower compartment respectively.
- k_1 and k_{-1} are rate constants for nanoparticle transport through the cell[3].

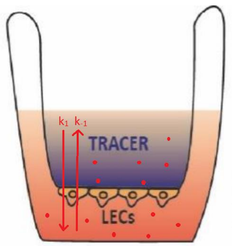

Figure 2: Lymphatic Transport Schematic

$$\frac{dn_{up}}{dt} = -k_1\, n_{up} + k_{-1}\, n_{lo} \quad \textbf{(1)}$$

$$\frac{dn_{lo}}{dt} = k_1\, n_{up} - k_{-1}\, n_{lo} \quad \textbf{(2)}$$

Methods

Artificial Neural Network

- A neural network with one hidden layer with 3 nodes was used **(Fig 3)**.
- The input layer has a single node since time is the only independent variable.
- The output layer has two nodes for the concentration in the upper and lower compartments.
- A backpropagation algorithm was used to fit the model to observed data, minimizing error between observed and expected results with each iteration.

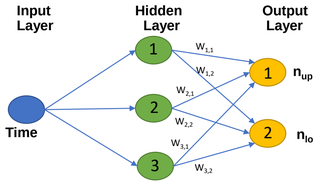

Figure 3: Artificial Neural Network Schematic

Results

Nanoparticle Surface Chemistry:

- To assess PEG density, we quantified the ratio of Flory radius (R_f) of the polymer to the measured grafting distance (D) **(Fig 4)**.

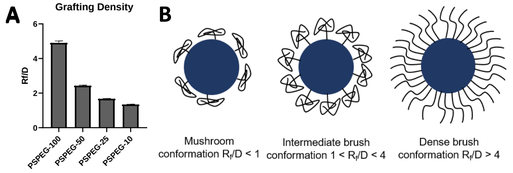

Figure 4: Nanoparticle Characterization
(A) Measured R_f/D values of differentially PEGylated NP correlated to the molar ratio of PEG to reactive carboxyl groups on the surface of the NP (n = 3).
(B) PEG added at different density generate nanoparticles with unique surface characteristics. R_f=Flory radius, D = grafting distance.

Nanoparticle Transport Efficiency

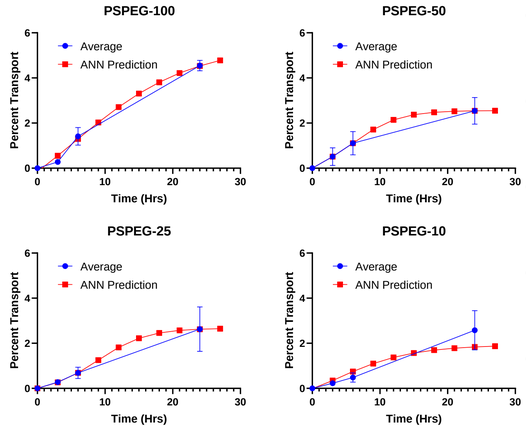

Figure 5: Effect of Surface Chemistry on Transport: Average percent transport over time is compared with neural network predictions for differentially pegylated nanoparticles.

- The in vitro model was used to assess transport efficacy of nanoparticles with varying surface chemistries.
- The artificial neural network was used to fit the data and extrapolate beyond measured time points **(Fig 5)**.
- Fully pegylated nanoparticles (PSPEG-100) in a dense brush conformation had the highest transport rates.
- Intermediate brush conformations (PSPEG-50 and PSPEG-25) had lower transport rates.
- Intermediate-mushroom conformation nanoparticles (PSPEG-10) had the lowest transport rates.

Results

Transport Inhibitors

- The impact of various inhibitors on nanoparticle transport across lymphatic endothelial cells was investigated **(Fig 6)**.
- Adrenomedullin strengthens tight junctions, which inhibits paracellular transport.
- Dynasore blocks endocytosis by inhibiting dynamic motor proteins.
- Both caused lower percent transport over time compared to a control.
- Lymphatic endothelial cells likely use both routes for nanoparticle transport.

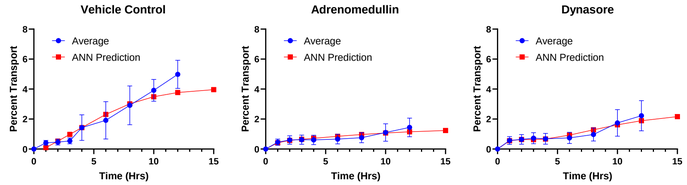

Figure 6: Effect of Transport Inhibitors Percent of PSPEG-100 NP transported after 24 hours under different transport inhibitors.

Conclusions

- A library of nanoparticles with different surface chemistries and PEG grafting densities was generated.
- Neutral, hydrophilic surface chemistry achieved through coating of nanoparticles with polyethylene glycol was found to improve lymphatic delivery.
- Highest transport rates across lymphatics resulted from fully pegylated nanoparticles in a dense brush surface conformation.
- The mechanisms through which lymphatic transport occurs – paracellular transport through tight junctions and endocytosis - were deciphered.
- An artificial neural network-based computational model was developed to allow for comparison between different nanoparticle formulations.
- As a future step, the model will be used to quantify the precise effects of surface chemistry on transport rates.
- The mass action laws will be expanded, with rate constants replaced by equations representing different pathways for transport.
- Knowledge gained in this study will inform nanoparticle design, which will be vital to improving the delivery, and therefore efficacy, of immunotherapies.

Acknowledgments

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